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Humidity Requirements for Mold Growth

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Received for publication June 22, 1953

In the warm, humid areas of the United States and of the world, mold or mildew prevention ranks as a major pest-control problem. Leather goods, clothing, foods, mattresses, painted walls, and even glass camera lenses are subject to the unsightly appearance, stain, damage, or musty odor associated with the growth of the lower-order plants belonging to the fungi and known popularly as mildew or mold.

As has been mentioned previously in a discussion of the mildew problem (Block, 1946), mold requires for growth and proliferation certain essential physical and chemical conditions. These include satisfactory temperature, adequate moisture, sufficient oxygen, proper pH, and essential nutrients. Prevention of mold growth may be effected by restricting any of the above requirements. Mildew may be controlled also by physical agents that destroy the organism, such as ultraviolet radiation and heat sterilization, and by chemical poisons called fungicides. The specific conditions best determine the method to be employed to prevent mold growth. In certain applications the control of moisture through regulation of humidity has proved to be most satisfactory (Block, 1951). This applies to the prevention of mildew in home closets, rooms, storage spaces and shipping units.

At the request of the British Public Record Office, Groom and Panisset (1933) studied the conditions necessary for mildew to develop on materials used for book covers. Working with *Penicillium chrysogenum*, they noted that the minimum relative humidity for spore germination on glass was 81 per cent and for mildewing of leather and other book materials was 72.8 per cent. Since it is obvious that mildew could not be present unless the mold spores could germinate,

Groom and Panisset suggest that the spores germinate at a lower relative humidity on book materials because of the presence of nutrients. Galloway (1935) reported that the minimum relative humidity permitting growth of molds varies from 75 to 95 per cent for different species; thus protection of materials is assured only if the atmosphere is below 75 per cent R.H. (relative humidity). Clayton (1942) found that some fungus spores germinate at 0 per cent humidity on glass, but these fungi are associated with plant diseases and not mildewing of inanimate objects. A report of the U. S. National Bureau of Standards (1947) states that at 85 per cent R.H., or less, no mildew growth on leather occurred, but that at 95 per cent R.H. there was heavy growth. Illman and Weatherburn (1947) made an excellent study of the factors affecting the development of mold on various materials. They found no mold growth at 60 per cent R.H. and very little at 70 per cent R.H., but increasing amounts from 80 to 100 per cent R.H.

To what extent does the moisture in the substrate determine mold growth as apart from the relative humidity of the atmosphere? How are different materials affected under the same physical conditions of humidity and temperature? Galloway (1935) offered data to show that atmospheric moisture is more effective than moisture in the substrata for bringing about the germination of mold spores. For information on the second question, Smith (1942) states, "If dry samples of pure wool and pure cotton are exposed to the same atmosphere the wool will take up approximately twice as much moisture as the cotton and, leaving out of account differences due to chemical composition, the two samples will be approximately equally liable to mildew."

According to Galloway (1935) the susceptibility of a material to mildew at a certain humidity is determined not by the nature of the material but by the type of fungi present. The following experimental work was begun in order to investigate and clarify further the relationship existing between humidity and the development of mildew.

EXPERIMENTAL

A number of materials differing widely in susceptibility to mildew and varying in their moisture-absorbing properties were selected for testing. These included leather (top shoe), cheese (American processed), wood (unpainted pine), wool (white flannel), cotton (heavy duck), and glass wool. The materials, where possible,

TABLE 1. Mixture of mold spores used for inoculating materials

1. *Aspergillus repens*
2. *Paecilomyces varioti*
3. *Rhizopus arrhizus*
4. *Penicillium namyslowskii*
5. *Aspergillus niger*
6. *Aspergillus fumigatus*
7. *Penicillium spinulosum*
8. *Aspergillus terreus*
9. *Penicillium oxalicum*
10. *Penicillium luteum*
11. *Penicillium pinophilum*
12. *Myrothecium verrucaria*
13. *Aspergillus flavus*
14. *Gliocladium fimbriatum*

TABLE 2. Saturated salt solutions employed to maintain constant humidity

RELATIVE HUMIDITY	SALT SOLUTION
100 per cent	H ₂ O
96 per cent	K ₂ SO ₄
92 per cent	NaH ₂ PO ₄
88 per cent	BaCl ₂
85 per cent	KCl
80 per cent	NH ₄ Cl
76 per cent	NaCl

were cut into thin sections one inch square and treated by spraying with mold spores in water or Czapek-Dox broth. The mold spores (table 1) were the mixture prepared by the American Leather Chemists Association¹ for testing mildew resistance of leather. There are arguments favoring either the use of a mixture of molds or a single mold species; however, it was felt that the mixture would be more suitable for these tests since it more closely represented natural conditions. The spore-inoculated pieces were air-dried. They were suspended by heavy cotton thread in quart Mason jars, in the bottoms of which were saturated solutions of different salts (table 2) to provide different but constant atmospheric humidities. Taking Galloway's figure of 75 per cent R.H. for the minimum relative humidity

¹ University of Cincinnati, Cincinnati, Ohio.

allowing mold growth, a series of humidities of 76, 80, 85, 88, 92, 96, and 100 per cent was used in tests of all the materials. The Mason jars were closed and kept at 85 to 90 F in the dark. The temperature was prevented from going below 85 F by a thermostated electric heater. At weekly intervals the samples were examined for the appearance of mildew. The extent of mold growth was estimated visually, employing an arbitrary scale from 0 to 5. Each material was tested both with and without

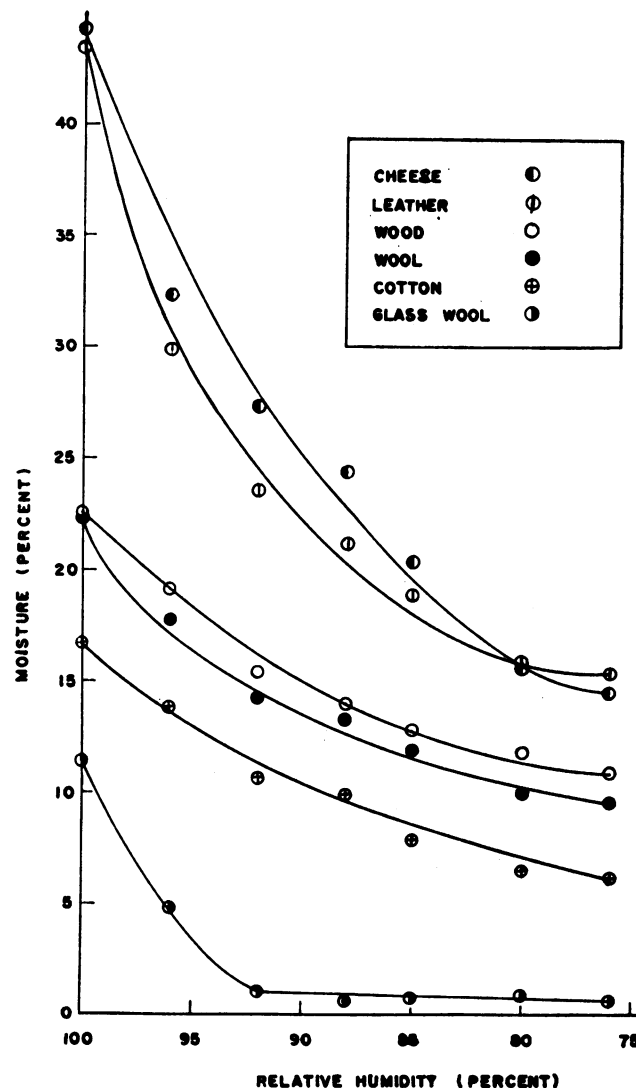


FIG. 1. The equilibrium moisture contents of several materials at 85 F and different relative humidities.

the added nutrients supplied by the Czapek-Dox broth.

The equilibrium moisture content of the materials at different humidities was determined by allowing the materials to remain at each humidity for several days at 85 F. The samples were then heated at 105 C for three hours and the percentage weight loss determined. The data are plotted in figure 1.

The moisture content of cotton duck was also varied by incorporating gum arabic and MgCl₂ into the cloth before exposure at various relative humidities.

TABLE 3. *Mold growth on different materials at different humidities with and without added nutrients*

TIME IN WEEKS	PER CENT HUMIDITY													
	100		96		92		88		85		80		76	
	Added Nutrients*	No Added Nutrients†	Added Nutrients	No Added Nutrients	Added Nutrients	No Added Nutrients	Added Nutrients	No Added Nutrients	Added Nutrients	No Added Nutrients	Added Nutrients	No Added Nutrients	Added Nutrients	No Added Nutrients
Leather														
1	3†	0	5	3	0	0	0	0	0	0	0	0	0	0
2	5	1	5	4	3	0	2	0	0	0	0	0	0	0
3	5	3	5	5	5	0	3	0	0	0	0	0	0	0
5	5	5	5	5	5	1	5	1	0	0	0	0	0	0
20	5	—	5	—	5	—	5	—	3	—	2	—	2	—
52	5	—	5	—	5	—	5	—	4	—	4	—	4	—
Cheese														
1	5	5	5	5	5	4	4	4	2	1	0	0	0	0
2	5	5	5	5	5	5	5	5	5	4	0	0	0	0
3	5	5	5	5	5	5	5	5	5	4	0	1	0	0
5	5	5	5	5	5	5	5	5	5	5	1	1	0	0
20	5	—	5	—	5	—	5	—	5	—	2	—	1	—
52	5	—	5	—	5	—	5	—	5	—	2	—	1	—
Wool														
1	3	T	3	T	0	0	0	0	0	0	0	0	0	0
2	4	4	4	T	0	0	0	0	0	0	0	0	0	0
3	5	4	5	1	0	0	0	0	0	0	0	0	0	0
5	5	5	5	1	3	0	0	0	0	0	0	0	0	0
20	5	—	5	—	3	—	1	—	1	—	0	—	0	—
52	5	—	5	—	4	—	4	—	1	—	0	—	0	—
Wood														
1	4	2	3	1	2	T	2	0	0	0	0	0	0	0
2	5	3	5	3	3	2	3	2	1	1	0	0	0	0
3	5	3	5	3	3	2	3	2	1	1	0	0	0	0
5	5	5	5	4	4	2	4	2	3	2	0	1	0	0
20	5	—	5	—	4	—	4	—	3	—	0	—	0	—
52	5	—	5	—	4	—	4	—	3	—	0	—	0	—
Cotton														
1	3	3	2	0	0	0	0	0	0	0	0	0	0	0
2	5	5	4	1	0	0	0	0	0	0	0	0	0	0
3	5	5	4	1	0	0	0	0	0	0	0	0	0	0
5	5	5	4	1	0	0	0	0	0	0	0	0	0	0
20	5	—	4	—	0	—	0	—	0	—	0	—	0	—
52	5	—	5	—	0	—	0	—	0	—	0	—	0	—
Glass Wool														
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	2	2	1	1	0	0	0	0	0	0	0	0	0	0
3	3	2	1	1	0	0	0	0	0	0	0	0	0	0
5	4	3	1	1	0	0	0	0	0	0	0	0	0	0
20	5	—	1	—	0	—	0	—	0	—	0	—	0	—
52	5	—	2	—	0	—	0	—	0	—	0	—	0	—

* Mold spore mixture (as prepared by the American Leather Chemists Association) in Czapek-Dox broth.

† Mold spore mixture (as prepared by the American Leather chemists Association) in water.

‡ Visual scale of mold growth: 0—None, T—Trace, 1—Very light, 2—Light, 3—Extensive, 4—Heavy, 5—Very heavy.

RESULTS

The mold growth on the different materials at different humidities at intervals over a period of one year

is given in table 3. Table 4 gives the collected data from table 3 comparing the mildew on different materials (with nutrients) after one year.

Table 5 demonstrates the influence of hygroscopic agents on the equilibrium moisture content of cotton and on the relative growth of mildew on this substrate. Table 6 gives data showing the effect of a fungicide on rate and extent of mold growth at different humidities. Figure 1 presents the equilibrium moisture contents of the materials at the humidities employed in these tests.

An examination of tables 3 and 4 shows that at 96 to 100 per cent R.H. all the materials were susceptible to mold growth. At 92 per cent R.H. and below, however, cotton and glass wool did not support mildew. At 80 per cent R. H. there was no growth of mold on wool

TABLE 4. *Mold growth on materials after one year at different humidities with added nutrients*

MATERIAL	PER CENT HUMIDITY						
	100	96	92	88	85	80	76
Leather.....	5*	5	5	5	4	4	4
Cheese.....	5	5	5	5	5	2	1
Wood.....	5	5	4	4	3	0	0
Wool.....	5	5	4	4	1	0	0
Cotton.....	5	5	0	0	0	0	0
Glass Wool.....	5	2	0	0	0	0	0

* Visual scale of mold growth: 0-None, T-Trace, 1-Very light, 2-Light, 3-Extensive, 4-Heavy, 5-Very heavy.

TABLE 5. *Effect of hygroscopic agents on mold growth on cotton at different humidities*

RELATIVE HUMIDITY	EQUILIBRIUM MOISTURE CONTENT OF COTTON			MOLD GROWTH IN 92 DAYS		
	Control	Gum Arabic	MgCl ₂	Control	Gum Arabic	MgCl ₂
	%	%	%			
100	25.9	27.8	42.0	5*	5	5
96	13.2	16.4	31.8	2	5	5
92	8.90	11.3	17.0	0	2	5
85	7.90	10.3	13.3	0	0	0

* Visual scale of mold growth: 0-None, T-Trace, 1-Very light, 2-Light, 3-Extensive, 4-Heavy, 5-Very heavy.

very slight on wood, light growth on cheese, and heavy growth on leather. At 76 per cent R.H., after a period of a year, there was very light growth on cheese but heavy growth on leather.

Observations on the rate of mold growth showed that cheese supported the most rapid growth, giving heavy growth in one week at as low as 88 per cent R.H. and in two weeks at 85 per cent R.H. Wood exhibited the next most rapid rate of growth, even though, as in the case of cheese, the extent of mold growth at the lower humidities was not so great as ultimately found with leather. The growth on glass wool was very slow, even at 100 per cent R.H., but at this humidity there was continuous growth until the sample was heavily covered

with mold. The rate of growth on leather was greater at 96 per cent R.H. than at 100 per cent R.H. This was regularly observed and might be explained by the presence of water droplets, on the waxy leather surface at 100 per cent R.H., interfering with the normal

TABLE 6. *The effect of a fungicide on the ability of mold to grow at different humidities*
Leather Substrate—o-phenyl phenol fungicide

CONCENTRATION OF FUNGICIDE	EXTENT OF MOLD GROWTH AT PER CENT HUMIDITY						
	100	96	92	88	85	80	76
5 days							
0.000	5*	3	0	0	0	0	0
0.01	5	2	0	0	0	0	0
0.10	4	0	0	0	0	0	0
1.00	0	0	0	0	0	0	0
11 days							
0.00	5	5	4	3	1	0	0
0.01	5	4	3	3	0	0	0
0.10	5	5	2	0	0	0	0
1.00	0	0	0	0	0	0	0
29 days							
0.00	5	5	4	4	3	0	0
0.01	5	5	4	4	2	0	0
0.10	5	5	4	4	1	0	0
1.00	0	0	0	0	0	0	0
51 days							
0.00	5	5	5	5	5	1	0
0.01	5	5	5	5	4	0	0
0.10	5	5	5	5	2	0	0
1.00	0	0	0	0	0	0	0
100 days							
0.00	5	5	5	5	5	2	1
0.01	5	5	5	5	4	1	0
0.10	5	5	5	5	3	1	0
1.00	0	0	0	0	0	0	0
130 days							
0.00	5	5	5	5	5	4	2
0.01	5	5	5	5	5	2	0
0.10	5	5	5	5	4	3	2
1.00	0	0	0	0	0	0	0

* Visual scale of mold growth: 0-None, T-Trace, 1-Very light, 2-Light, 3-Extensive, 4-Heavy, 5-Very heavy.

growth of the mold. At 96 per cent R.H. there were no droplets of water collecting on the samples. Except for the leather and glass wool, the materials were sufficiently porous to prevent the accumulation of water droplets.

The effect of the added nutrients on the rate of mold

growth on the different materials is presented in table 3. With cheese there was very little difference in the rate of growth, indicating, as would be expected, an abundance of readily available nutrients. All of the other materials showed a definite increase in rate of mold growth where additional nutrients were provided.

Hygroscopic agents added to cotton resulted in higher moisture content and mold growth at lower humidities than in the untreated cotton (table 5). Gum arabic provides, as well, a source of organic carbon which could serve as nutrient for the mold, but that is not true of magnesium chloride.

At the minimum effective concentration of fungicide, the rate of mold growth was decreased. However, the ultimate amount of growth obtained with extended incubation was not changed. The fungicide did not alter the minimum humidity at which mold growth occurred (table 6).

DISCUSSION

If the temperature and other necessary conditions are favorable for mold growth, what is the maximum humidity below which materials must be maintained in order to keep them mildew-free? This is a practical problem frequently encountered where materials are stored or shipped. From the data in tables 3 and 4, it is clear that the "safe" humidity will depend upon the material under consideration. Cotton fabric can be kept free of mildew at 92 per cent R.H., while leather and cheese must be stored below 76 per cent R.H. If the storage time is shorter than the incubation periods noted in table 3 for different humidities, the materials may be safely kept at a humidity which would ultimately permit mold growth. Removal of nutrients and hygroscopic agents (as occurs, for example, in the washing or dry cleaning of clothing) or use of chemical mildew inhibitors can increase the safe storage time by retarding mold growth and increasing the incubation period. From the experiments described here and those given in the literature, at atmosphere of 65 per cent R.H. or less, might be considered safe for permanent storage of all materials.

Examination of figure 1 and table 4 demonstrate that the materials which are most hygroscopic are most susceptible to mold growth and vice versa. It is true, as Smith (1942) has stated, that if pure cotton and pure wool are exposed to the same atmosphere, the wool will take up more moisture, but, differing from Smith, the two materials were found to be not equally liable to mildew. Wool is much more liable to mildew, and this is found to be true in practice as well as in laboratory experiments. That these differences are not due to the presence or absence of nutrients has been shown by the ready mildewing of the materials at 100 per cent R.H. and the comparative results when nutrients were added to the materials. The mold is apparently able to secure

for itself moisture taken from the air by the substrate. The greater the quantity of water absorbed by the substrate, the more water becomes available to the mold.

Galloway's conclusion (1935) that the humidity at which mild growth occurs is determined by the nature of the fungi present, and not by the nature of the material, has not been confirmed in this work. The large representation of fungus spores with which all the materials were inoculated (table 1), in addition to the natural flora of the materials (the samples were not sterilized), assured that mildew would have appeared on all materials at the different humidities tested if the nature of the fungi present were the only factor involved. There can be no question that spores capable of germination at a specified humidity must be present for mildew to occur, but evidence that spores germinate at a specified humidity is not necessarily proof that mold growth will take place. Spore germination and mycelial proliferation are not identical physiological processes.

Reference to figure 1 and table 4 indicates that the minimum equilibrium moisture content for the occurrence of mildew on all materials, except glass wool varied from about 10 per cent to about 14 per cent moisture. Although these data are not available for leather and cheese, which mildewed at the lowest humidity tested, the curves can be extrapolated to fall into the same general moisture range as found for the other materials. O'Flaherty and Doherty (1940) found no mold growth on leather having less than 14 per cent moisture. Glass wool may be exceptional because it is not absorptive, and the moisture is concentrated at the surface of the fibers. If the "safe" equilibrium moisture content of 12 ± 2 per cent holds generally for all materials, the potential susceptibility of new materials to mildew may be determined by measuring the equilibrium moisture content of the new material at the humidity at which it is to be kept. If this figure is below 10 per cent, it is likely that the material will not mildew, whereas if it is above 14 per cent, the material will mildew if all the other requirements for mold growth are satisfied.

In tests of a number of different materials used as book covers, Groom and Panisset (1933) found none of the materials mildewed when their moisture content was below 10 per cent. With some materials, no mildew appeared even though the moisture content was almost 80 per cent. In such cases it can be assumed that factors other than insufficient moisture prevented mold growth. It has been stated that moisture is a controlling factor for mold growth, providing other requirements are fulfilled. Under unfavorable conditions mold growth may occur only at a higher relative humidity than is required under optimum conditions. Whereas the data in this paper appear to indicate that nutrients and inhibitors affect essentially the rate of growth and not

the minimum humidity for mold growth, the rate is, in practice, the controlling factor where the lag phase of growth is extended considerably by inhibitors or lack of nutrients. Thus a low concentration of a fungicide that is ineffective at a high relative humidity may so delay the appearance of mold at a lower relative humidity (table 6) that, for practical purposes, there is no mildew problem.

Prindle (1937), working with raw cotton, was able to detect growth of *Aspergillus* and *Penicillium* at 82 per cent R.H. For raw cotton, this corresponds to about 10.5 per cent moisture. No musty mold odor was observed below 86 per cent R.H., or about 12 per cent moisture. Cotton mattresses contain raw cotton and exhibit the unpleasant musty odor when the relative humidity is about 85 per cent. It is evident that raw cotton differs from the cotton fabric used in these tests. Nevertheless the minimum percentage moisture for the occurrence of mildew was the same. Armstead and Harland (1923) obtained no mold on cotton cloth at 90 per cent R.H. (7.0 per cent H₂O), moderate growth at 92 per cent R.H. (7.8 per cent H₂O) and profuse growth at 94 per cent R.H. (14.8 per cent H₂O). With different samples of cotton cloth, Galloway (1930) found no mildew at 9 per cent moisture and only very slight mildew where the cloth contained 9.5 or 10.0 per cent moisture. The raw cotton tested by Prindle (1937) contains moisture-absorbing substances which are removed in processing. In a like manner, Burgess (1929) observed that wool which was treated with soap mildewed at 91 per cent R.H. while untreated wool mildewed only at 97 per cent R.H. and above. In table 3 of this report it will be noted that in five weeks there was growth on nutrient-treated wool at 92 per cent R.H., but on untreated wool growth occurred only at 96 per cent R.H. and above. These results are practically identical with those of Burgess (1929). In a period of 20 weeks, however, growth was observed on wool at 85 per cent R.H. It will be noted from table 5, which shows the effect of added hygroscopic agents, that mold failed to appear where the moisture content was below 10 per cent but did grow in all cases where the moisture content was above 14 per cent. It was observed by Galloway (1935) that magnesium chloride stimulated germination of mold spores, and he attributed to this factor, rather than to increased moisture, the growth of mold at lower humidities in the presence of deliquescent agents. The parallel effect that was obtained with gum arabic and the fact that spores germinated on other materials at lower humidities, providing the moisture content of the material was above 14 per cent, suggest that moisture rather than spore germination was the limiting factor. The results indicate that the molds obtain their moisture directly from the substrate rather than from the moisture in the air.

Under nonequilibrium moisture conditions, the re-

sults obtained may be different from those which have been reported in this paper. If a material containing a high concentration of moisture is placed in an atmosphere which has a low relative humidity, there is a continual transfer of moisture from the substrate to the atmosphere until an equilibrium is established. Under such transitory conditions, many factors are involved and the problem becomes very difficult to study. Actually, nonequilibrium conditions, resulting from movement of air and temperature fluctuations are, in practice, the rule rather than the exception. In a home, the warm midday air dries surfaces which it contacts and helps to prevent mold growth in the rooms. In the closets and storage spaces, where this air has no ready access, mildew is much more prevalent. The effect of ventilation in retarding mildew is one of drying, for our experiments have shown that air motion, by itself, does not retard mold growth. Under natural conditions, as in a home closet, the appearance of mildew occurs at a more rapid rate than under approximately the same average relative humidity in the jars employed in this experiment. The fresh supply of oxygen and removal of carbon dioxide and other inhibitory gases under natural conditions may explain the more rapid rate of mold proliferation. Nevertheless, observation of mold under natural conditions verifies the results obtained in these experiments for the mildewing of different materials at specified minimum humidities. Mildewing of leather is most prevalent, followed by wooden and woolen articles, and by cotton and glass in decreasing order of prevalence.

CONCLUSIONS

The more hygroscopic a material, the lower the relative humidity at which it was found capable of supporting mold growth. In humidity tests of one year's duration, leather and cheese were susceptible to growth of mildew at 76 per cent R.H. and higher; wood and wool mildewed at 85 per cent R.H. and greater, while cotton cloth and glass wool failed to mildew at 92 per cent R.H. but mildewed at 96 per cent and 100 per cent R.H.

The rate of mold growth was stimulated by additional nutrients and retarded by a fungicide, but the ultimate extent of mold growth and the minimum humidity at which the material was susceptible did not appear to be appreciably affected. The marked increase in the rate of mold growth in the presence of nutrients emphasizes the importance of cleanliness in preventing mildew, particularly of materials like cotton and wool that offer no inherent nutrition for most common molds.

The minimum humidity for the occurrence of mold growth was related to the equilibrium moisture content of each material at each humidity. Except for glass wool, all materials could be said to have their minimum moisture contents for growth of molds in the range of

approximately 12 ± 2 per cent. Hygroscopic agents added to cotton cloth raised its equilibrium moisture content and lowered the relative humidity at which it mildewed.

It is concluded that the water-absorbing properties of the substrate play an all-important role in determining the limiting humidity of the atmosphere at which mildew will occur. It is postulated that the fungus is incapable of obtaining moisture for mycelial development directly from the atmosphere (except at 100 per cent R.H.) but derives it from the substrate which obtains the moisture from the atmosphere.

Under natural conditions the more rapid proliferation of mold may be attributed to the more plentiful supply of oxygen and the constant removal of toxic gases. In general, however, observations of the occurrence of mildew under natural conditions support the findings reported herein.

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Some Variables in The Assay of Bacitracin

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Received for publication July 3, 1953

The zone sizes of a given preparation of bacitracin were found to vary sufficiently in a series of agar plate assays to warrant investigation. Many factors have been found to influence the zone sizes of antibiotics. Variations in the assay of penicillin have been demonstrated in connection with the depth of the agar, the position of the assay plates during incubation, and the original temperature of the agar plates (Cooper and Linton, 1952). De Beer and Sherwood (1945) have demonstrated variations due to heavy seeding of the test plates, and Loo, *et al.*, (1945) have described the differences obtained with the use of cylinders and filter paper discs.

An agar diffusion assay for bacitracin with *Micrococcus flavus* was described by Hoff, Bennett and Stanley (1947). Subsequent modifications led to the F.D.A. assay procedure which was used in these investigations.

It appeared that the variations in the bacitracin

assays were related to various delays encountered in the assay procedure, and these factors were subjected to further study.

EXPERIMENTAL METHODS

F.D.A. assay procedures were followed for each of the antibiotics used in this study. Plates were poured with a base layer of agar and a seeded overlay. Six stainless steel cylinders were placed on the surface of each plate by means of a Shaw¹ cupsetter. Three alternate cups were filled with a solution of the antibiotic under study. Half the plates were held at room temperature, while the other half were placed at refrigeration temperature (4 C). At various time intervals, ranging from $\frac{1}{4}$ to 8-hours, the antibiotic solutions used previously were added to the remaining 3 cups on duplicate plates from the two groups. The plates were then incubated at 37 C for the specified time

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